

# Disease Exposure and Antifungal Bacteria on Skin of Invasive Cane Toads, Australia

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Cane toads, an invasive species in Australia, are resistant to fungal pathogens affecting frogs worldwide (*Batrachochytrium dendrobatidis*). From toad skin swabs, we detected higher proportions of bacteria with antifungal properties in Queensland, where toad and pathogen distributions overlap, than in other sites. This finding suggests that site-specific pathogen pressures help shape skin microbial communities.

The westward and southward spread of invasive cane toads (*Rhinella marina*) in Australia since their introduction to Queensland in 1935 threatens many native species. In addition to their skin-secreted bufotoxins affecting predators, toads are resistant to the fungal pathogen *Batrachochytrium dendrobatidis* associated with global frog die-offs, but their capacity to spread the pathogen to other frog species remains unclear (1,2).

As a skin pathogen, *B. dendrobatidis* interacts not only with the host's immune system, but also with other community members in the skin microbiome (3). Many bacteria on frog skin have antifungal properties that can help the host fight *B. dendrobatidis* (4), and the presence of bacteria with anti-*B. dendrobatidis* capacity may increase a host's pathogen resistance. In a previous study about gene expression in experimentally infected cane toads, their strong resistance to *B. dendrobatidis* was not attributed to strong immune function (1). Thus, the skin environment, including microbes inhabiting it, alongside an immediate, localized immune response, might play a large role in fighting the pathogen and resisting disease (1).

Invading species are predicted to invest in less costly immune defenses while decreasing their investment in costly inflammatory immune responses (5). With the assumption that skin bacteria are relatively inexpensive for the host to maintain, we used skin swab samples collected in 2017 to test whether cane toads have increased

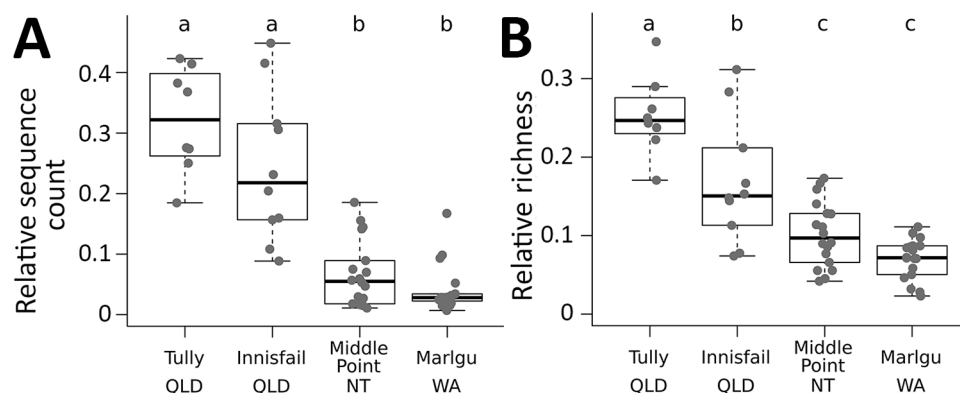
proportions of putative *B. dendrobatidis*-inhibiting bacteria at the invasion front in Australia, consistent with a previously reported increased investment into low-cost innate immune functions (6). Alternatively, we predicted that patterns of *B. dendrobatidis*-inhibitory bacteria on toad skin might depend on the current distribution of, and thus likely exposure to, *B. dendrobatidis*. Our 4 sampling locations (8–18 per site; Appendix Figure 1, <https://wwwnc.cdc.gov/EID/article/25/9/19-0386-App1.pdf>) comprised 2 sites overlapping the current *B. dendrobatidis* distribution (Queensland) and 2 sites outside the area of threat of chytridiomycosis (Northern Territory and Western Australia). These 4 sites also represent the toad's westward expansion; Western Australian toads were sampled near the invasion front. Sampling was in accordance with Charles Darwin University Animal Ethics permit A14012.

We compared bacterial amplicon sequence variants (sequences available on FigShare, <https://doi.org/10.6084/m9.figshare.7855670>) against a database of known anti-*B. dendrobatidis* isolates from the skin of frog species around the world (4). We detected 63 bacterial types with previously described anti-*B. dendrobatidis* function in the wild toad samples in our study. The 4 sampling sites differed in the proportion of total sequences and bacterial types represented by putative *B. dendrobatidis*-inhibitory bacteria, and toads from Queensland sites had proportionately more of these sequences and taxa than did toads from other sites (Figure). Some of the *B. dendrobatidis*-inhibitory bacteria were extremely common among our samples (particularly at the Tully site in Queensland [Appendix]).

Our results indicate that the skin bacterial communities on toads from sites overlapping the *B. dendrobatidis* distribution contain more putative *B. dendrobatidis*-inhibitory bacteria than is the case for toads from sites not yet invaded by the pathogen. Rather than following predictions regarding immunocompetence at the invasion front, this pattern suggests that *B. dendrobatidis*-inhibitory bacteria are selected for where the disease is present, in concordance with the adaptive microbiome hypothesis presented by Jin Song et al. (7). Outside of the *B. dendrobatidis* range, selection for anti-*B. dendrobatidis* microbes is relaxed; inhibitory microbes represent less of the community, and some disappear.

In cane toads, juvenile life stages succumb to *B. dendrobatidis*, although they have higher survival rates and better ability to clear an infection than other amphibians (e.g., 1). The prevalence of bacteria with *B. dendrobatidis*-inhibitory capacity on adult cane toads in Queensland suggests that the skin microbiome might confer some of the resistance to disease in this host species. Although amphibian skin microbiome communities change across ontogeny, host species is a strong predictor of skin communities

**Figure.** Proportions of sequences (A) and richness (B) represented by *Batrachochytrium dendrobatidis*–inhibitory bacteria detected on the skin of invasive cane toads (*Rhinella marina*) at 4 sites in Australia, 2017. Points indicate values for individual toads. Box plots indicate the median (thick line), interquartile range (box), reasonable range of the data (dashed lines to the whiskers), and outliers. Letters above plots indicate significant differences from Tukey's post hoc tests with  $p < 0.05$ . QLD, Queensland; NT, Northern Territory; WA, Western Australia.



across life stages (8). Thus, the communities found on these adult toads may predict those found on juvenile toads.

Our results could be affected by larger differences in bacterial community composition that can occur among sampling sites in cane toads (9) and other frog species (8). These differences could be due to diverse environmental microbiota supported by climatic and other abiotic and biotic conditions that change across the landscape.

The detection of *B. dendrobatidis*–inhibitory microbes at *B. dendrobatidis*–naïve sites might be misleading. The presence of a functional gene does not necessarily imply gene activity (10). Thus, the approach of ascribing *B. dendrobatidis*–inhibitory roles based on presence might be too simplistic in the absence of direct evidence of *B. dendrobatidis*–inhibitory action, which was outside the scope of this study. Some of these bacteria may be commonly found on cane toad skin, regardless of an active function to inhibit *B. dendrobatidis*. Nonetheless, the higher frequency of these bacteria in *B. dendrobatidis*–exposed locations suggests that the microbiome on the skin of cane toads is shaped, at least partly, by natural selection in response to geographic variation in the degree of threat posed by specific diseases.

### Acknowledgments

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### About the Author

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### References

- Poorten TJ, Rosenblum EB. Comparative study of host response to chytridiomycosis in a susceptible and a resistant toad species. *Mol Ecol*. 2016;25:5663–79. <https://doi.org/10.1111/mec.13871>
- Brannelly LA, Martin G, Llewelyn J, Skerratt LF, Berger L. Age- and size-dependent resistance to chytridiomycosis in the invasive cane toad *Rhinella marina*. *Dis Aquat Organ*. 2018;131:107–20. <https://doi.org/10.3354/dao03278>
- Jani AJ, Briggs CJ. The pathogen *Batrachochytrium dendrobatidis* disturbs the frog skin microbiome during a natural epidemic and experimental infection. *Proc Natl Acad Sci U S A*. 2014;111:E5049–58. <https://doi.org/10.1073/pnas.1412752111>
- Woodhams DC, Alford RA, Antwis RE, Archer H, Becker MH, Belden LK, et al. Antifungal isolates database of amphibian skin-associated bacteria and function against emerging fungal pathogens. *Ecology*. 2015;96:595–595. <https://doi.org/10.1890/14-1837.1>
- Lee KA, Klasing KC. A role for immunology in invasion biology. *Trends Ecol Evol*. 2004;19:523–9. <https://doi.org/10.1016/j.tree.2004.07.012>
- Brown GP, Phillips BL, Dubey S, Shine R. Invader immunology: invasion history alters immune system function in cane toads (*Rhinella marina*) in tropical Australia. *Ecol Lett*. 2015;18:57–65. <https://doi.org/10.1111/ele.12390>
- Jin Song S, Woodhams DC, Martino C, Allaband C, Mu A, Javorschi-Miller-Montgomery S, et al. Engineering the microbiome for animal health and conservation. *Exp Biol Med* (Maywood). 2019;244:494–504. <https://doi.org/10.1177/1535370219830075>
- Kueneman JG, Parfrey LW, Woodhams DC, Archer HM, Knight R, McKenzie VJ. The amphibian skin-associated microbiome across species, space and life history stages. *Mol Ecol*. 2014;23:1238–50. <https://doi.org/10.1111/mec.12510>
- Christian K, Weitzman C, Rose A, Kaestli M, Gibb K. Ecological patterns in the skin microbiota of frogs from tropical Australia. *Ecol Evol*. 2018;8:10510–9. <https://doi.org/10.1002/ece3.4518>
- Wang Y, Zhang R, He Z, Van Nostrand JD, Zheng Q, Zhou J, et al. Functional gene diversity and metabolic potential of the microbial community in an estuary-shelf environment. *Front Microbiol*. 2017;8:1153. <https://doi.org/10.3389/fmicb.2017.01153>

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# Disease Exposure and Antifungal Bacteria on Skin of Invasive Cane Toads, Australia

## Appendix

### Sample Collection and Microbiome Sequencing

In September 2017, cane toads (*Rhinella marina*) were swabbed for skin-associated bacteria at 4 sampling sites in Australia: Tully, Queensland (n = 8); Innisfail, Queensland (n = 10); Middle Point, Northern Territory (n = 18); and Marlgu Billabong, Western Australia (n = 18), near the cane toad invasion front (Appendix Figure 1). Free-ranging toads were caught by hand or net and rinsed with 100 mL 0.45 µm high-purity water (1,2) before collecting swab samples with a sterile synthetic swab (Transwabs, Cat # MW167S; Medical Wire and Equipment Company, <https://www.mwe.co.uk>). Swabbing included 30 strokes around the entire toad body, excluding the cloaca and head, following protocols in Christian et al. (3). Toad handlers wore new gloves for each swabbed animal. Swab samples were placed on ice in the field before frozen at –20°C.

DNA was extracted from swab samples using the Norgen's Swab Collection & DNA Preservation Kit (Cat # 45681, Norgen Biotek Corp., <https://norgenbiotek.com>) following the protocol. Extracted DNA was quantified, and we sent 200 ng of dried DNA to ACE Sequencing Service at the Australian Centre for Ecogenomics (University of Queensland) for Illumina MiSeq sequencing (Illumina, <https://www.illumina.com>).

Sequencing targeted the V4 region of small subunit rDNA using F515/R806 primers (4). Sequencing was conducted on multiplexed samples, which included additional toad swab samples and 2 negative controls based on DNA extracted from clean swabs. Sequences were processed to amplicon sequence variants (ASVs) by ACE with the following pipeline. The software Trimmomatic was used for sequence quality trimming removing poor quality sequences with a sliding window of 4 bases and an average base quality >15. All reads were hard trimmed to 250 bases, and any shorter reads were excluded. Reads were processed to ASVs using the

QIIME2 workflow with default parameters and the DADA2 algorithm (5,6). Taxonomic assignment of ASVs was through BLAST+ using the reference database SILVA (<https://www.arb-silva.de>). Only ASVs identified as Bacteria were kept. Bacterial ASVs and associated metadata are available on FigShare: <https://doi.org/10.6084/m9.figshare.7855670>.

After inspecting rarefaction curves, we subsampled data to the lowest sequence count (2,744) per toad sample, corresponding with 41 to 311 bacterial types per sample.

We used QIIME2 to compare feature sequences against a database of isolates whose fungal inhibitory capacity have previously been tested against the fungal pathogen, *Batrachochytrium dendrobatidis* (7). The Antifungal Isolates Database contains information on the amphibian host species the bacterium was isolated from, geographic location where the host was caught and sampled, fungal-inhibition results, and bacterial taxonomy from UClust (8) and RDP (9). The database contains information on >1,100 bacterial isolates with capacity for *B. dendrobatidis*-inhibition.

We used the “cluster-features-closed-reference” option to identify feature sequences in our dataset that were at least 99% similar to sequences in the Woodhams et al. database (7). Resultant data were then separated into *B. dendrobatidis*-inhibitory and *B. dendrobatidis*-enhancing bacteria, and we determined sequence count and richness per sample. Because negative controls had sequence counts too low to be retained after rarefaction (<600 sequences each), we compared negative control data against the Woodhams et al. database separately for qualitative purposes. Lastly, we identified which *B. dendrobatidis*-inhibitory bacterial types were present in the majority of samples from each sampling site (often referred to as “core” microbiome; 87.5% prevalence cutoff).

## Statistical Analyses

We used Kruskal-Wallis tests to compare richness and Shannon diversity of the rarefied microbiome communities to detect inherent differences among the sites. Similar to analyses in Varela et al. (10), we compared the proportion of total sequences or richness represented by *B. dendrobatidis*-inhibitory bacteria using generalized linear models. Inhibitory data were analyzed using a quasibinomial distribution, with richness proportions weighted for total richness. *B. dendrobatidis*-enhancing sequence count data were analyzed using a quasipoisson distribution,

because the values were small (maximum = 5.3% of sequences) and showed overdispersion. Tukey's post-hoc tests were used for pairwise site comparisons using the `glht` function in the `multcomp` R package (11). All analyses were conducted in R v. 3.4.4 (12).

## Results

We detected 63 *B. dendrobatidis*–inhibitory bacterial types in our samples, with 4 to 23 per sample. The negative controls had 2 *B. dendrobatidis*–inhibitory bacterial types, 1 of which was not present in our wild toad samples. The second, *Stenotrophomonas* sp. (Phylum: Proteobacteria), was present in wild toad samples and was a “core” OTU in Tully samples (Appendix Table). Known *B. dendrobatidis*–inhibitory bacteria represented up to 45% of the total sequences in a sample. Most of the *B. dendrobatidis*–inhibitory bacteria were originally isolated from amphibians in Latin America, with additional bacteria from the United States and Madagascar. Nine were isolated from *Litoria* spp. of frogs from Queensland, Australia.

From Kruskal-Wallis tests, we did not find significant differences in richness ( $\chi^2 = 6.2061$ ,  $df = 3$ ,  $p = 0.102$ ) or Shannon's diversity index ( $\chi^2 = 5.242$ ,  $df = 3$ ,  $p = 0.1549$ ) among our sites.

Generalized linear models found significant differences among sites in relative *B. dendrobatidis*–inhibitory sequence count and richness: proportion inhibitory sequences,  $F_{(3,50)} = 35.34$ ,  $p < 0.0001$ ; proportion inhibitory richness,  $F_{(3,50)} = 26.24$ ,  $p < 0.0001$ . From Tukey's post hoc tests for pairwise comparisons between sites, toads from Queensland sites had a greater proportion of sequences and bacterial types represented by *B. dendrobatidis*–inhibitory bacteria (Figure).

Of the 63 *B. dendrobatidis*–inhibitory bacteria in our samples, 14 were in the “core” microbial community (microbes with 87.5%+ prevalence) of one or more geographic sampling sites. The Tully site in Queensland had 13 known *B. dendrobatidis*–inhibitory bacteria in its core community, which was likely influenced both by the site's small sample size of 8 and the presence of chytridiomycosis in the region. The other sites had only 2 to 5 *B. dendrobatidis*–inhibitory bacterial types in their core microbiota. Three *B. dendrobatidis*–inhibitory bacteria were commonly found on toads from all 4 sites sampled in our study (in  $\geq 46$  of our 54 samples): *Sphingobacterium multivorum* (Phylum Bacteroidetes), originally isolated from a Waterfall Frog

in Queensland; a *Microbacteriaceae* (Phylum Actinobacteria), originally isolated from an American Bullfrog in Colorado, USA; and an *Enterobacteriaceae* (Phylum Proteobacteria), originally isolated from a *Bufo typhonius* toad in Panama. The Appendix Table contains a list of the bacteria found in at least 87.5% of the samples per site.

Our cane toad samples had only 2 *B. dendrobatidis*-enhancing bacteria from the Woodhams et al. 2015 database (7), both originally isolated from Panamanian frogs: *Acinetobacter rhizopherae* (Phylum Proteobacteria) and *Microbacterium* sp. (Phylum Actinobacteria). *B. dendrobatidis*-enhancing bacteria represented up to  $\approx 5\%$  of the total sequences and richness in a sample. From generalized linear models, sites were found to differ in proportion of *B. dendrobatidis*-enhancing sequences ( $F_{(3,50)} = 6.08$ ,  $p = 0.001$ ). Some pairwise differences were detected between sites, with toads from the Northern Territory having greater abundance of *B. dendrobatidis*-enhancing bacteria than toads from both Queensland sites (Appendix Figure 2). The Woodhams et al. database (7) contains only 58 known *B. dendrobatidis*-enhancing isolates, none of which were isolated from Australian species. The database was compiled to identify bacteria that may aid in the fight against the pandemic frog chytridiomycosis, and as such, is skewed toward antifungal, and not fungal-enhancing bacteria. Nevertheless, in the face of this disease, the presence of *B. dendrobatidis*-enhancing bacteria representing nearly 5% of the bacteria on a disease-resistant, invasive cane toad's skin could be an important consideration when seeking to understand chytridiomycosis on native Australian frogs.

## References

1. Culp CE, Falkinham JO III, Belden LK. Identification of the natural bacterial microflora on the skin of eastern newts, bullfrog tadpoles and redback salamanders. *Herpetologica*. 2007;63:66–71. [https://doi.org/10.1655/0018-0831\(2007\)63\[66:IOTNBM\]2.0.CO;2](https://doi.org/10.1655/0018-0831(2007)63[66:IOTNBM]2.0.CO;2)
2. Lauer A, Simon MA, Banning JL, André E, Duncan K, Harris RN. Common cutaneous bacteria from the eastern red-backed salamander can inhibit pathogenic fungi. *Copeia*. 2007;2007:630–40. [https://doi.org/10.1643/0045-8511\(2007\)2007\[630:CCBFTE\]2.0.CO;2](https://doi.org/10.1643/0045-8511(2007)2007[630:CCBFTE]2.0.CO;2)
3. Christian K, Weitzman C, Rose A, Kaestli M, Gibb K. Ecological patterns in the skin microbiota of frogs from tropical Australia. *Ecol Evol*. 2018;8:10510–9. [PubMed](https://doi.org/10.1002/ece3.4518) <https://doi.org/10.1002/ece3.4518>

4. Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Lozupone CA, Turnbaugh PJ, et al. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. *Proc Natl Acad Sci U S A*. 2011;108(Suppl 1):4516–22. [PubMed](#) <https://doi.org/10.1073/pnas.1000080107>
5. Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, et al. QIIME allows analysis of high-throughput community sequencing data. *Nat Methods*. 2010;7:335–6. [PubMed](#) <https://doi.org/10.1038/nmeth.f.303>
6. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. DADA2: High-resolution sample inference from Illumina amplicon data. *Nat Methods*. 2016;13:581–3. [PubMed](#) <https://doi.org/10.1038/nmeth.3869>
7. Woodhams DC, Alford RA, Antwis RE, Archer H, Becker MH, Belden LK, et al. Antifungal isolates database of amphibian skin-associated bacteria and function against emerging fungal pathogens. *Ecology*. 2015;96:595–595. <https://doi.org/10.1890/14-1837.1>
8. Edgar RC. Search and clustering orders of magnitude faster than BLAST. *Bioinformatics*. 2010;26:2460–1. [PubMed](#) <https://doi.org/10.1093/bioinformatics/btq461>
9. Wang Q, Garrity GM, Tiedje JM, Cole JR. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Appl Environ Microbiol*. 2007;73:5261–7. [PubMed](#) <https://doi.org/10.1128/AEM.00062-07>
10. Varela BJ, Lesbarrères D, Ibáñez R, Green DM. Environmental and host effects on skin bacterial community composition in Panamanian frogs. *Front Microbiol*. 2018;9:298. [PubMed](#) <https://doi.org/10.3389/fmicb.2018.00298>
11. Hothorn T, Bretz F, Westfall P. Simultaneous inference in general parametric models. *Biom J*. 2008;50:346–63. [PubMed](#) <https://doi.org/10.1002/bimj.200810425>
12. R Core Team. R: a language and environment for statistical computing. Vienna (Austria): R Foundation for Statistical Computing; 2014 [cited 2019 Jun 21]. <http://www.R-project.org>.



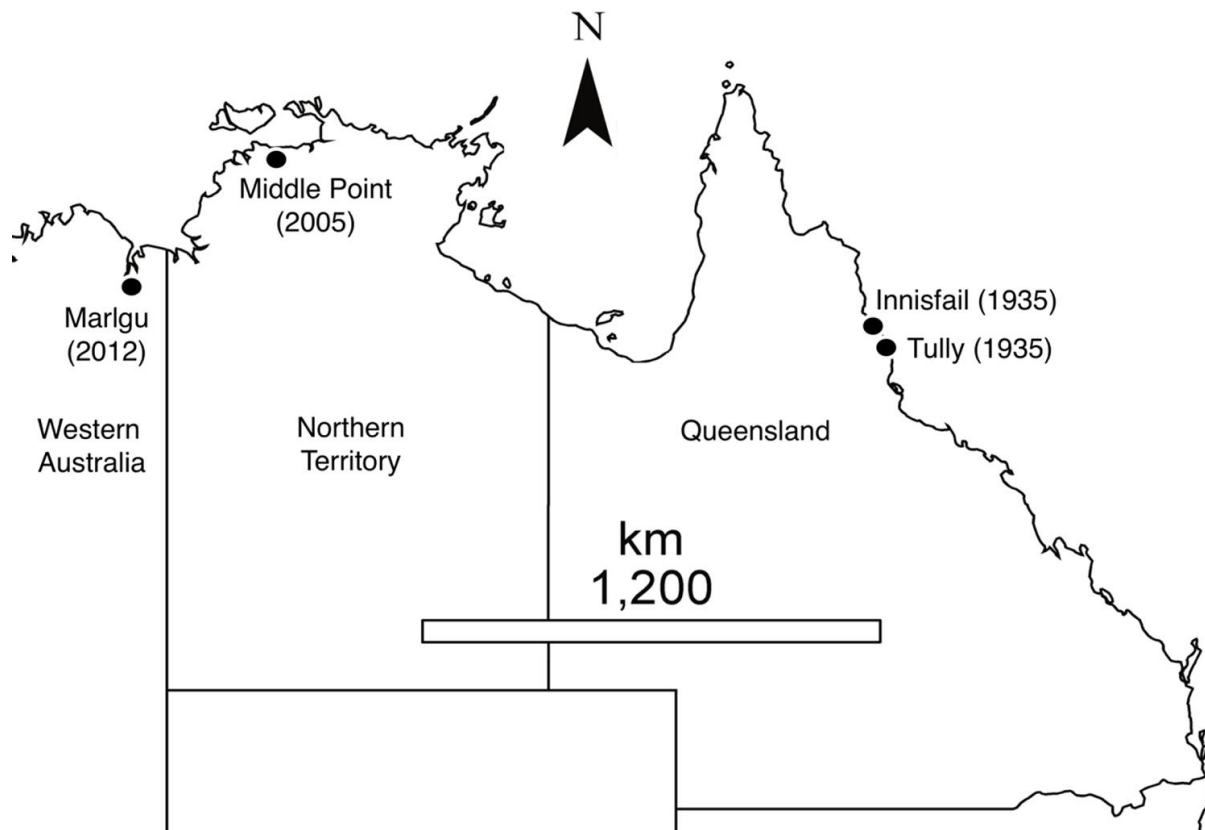
**Appendix Table.** Core *Batrachochytrium dendrobatidis*-inhibitory bacteria on wild cane toads by sampling site, Australia, 2017\*

SampleID	TULLY, n = 8	INN, n = 10	NT, n = 18	WA, n = 18	Region	Uclust_taxonomy
Atelopuslimosus-inhibitory_28	8	9			Panama	p__Proteobacteria; c__Gammaproteobacteria; o__Pseudomonadales; f__Moraxellaceae; g__Acinetobacter; s__
Atelopuslimosus-inhibitory_64	7				Panama	p__Bacteroidetes; c__Flavobacteriia; o__Flavobacteriales; f__[Weeksellaceae]; g__Chryseobacterium; s__
Bufo typhoni-us-inhibitory_9	7	9		16	Panama	p__Proteobacteria; c__Gammaproteobacteria; o__Enterobacteriales; f__Enterobacteriaceae
Craugastorcrassidigitus-inhibitory_109	8	9			Panama	p__Proteobacteria; c__Betaproteobacteria; o__Burkholderiales; f__Comamonadaceae; g__Comamonas; s__
Craugastorcrassidigitus-inhibitory_121	8	9			Panama	p__Proteobacteria; c__Gammaproteobacteria; o__Pseudomonadales; f__Pseudomonadaceae; g__Pseudomonas; s__
Craugastorcrassidigitus-inhibitory_165	7			17	Panama	p__Bacteroidetes; c__Flavobacteriia; o__Flavobacteriales; f__[Weeksellaceae]; g__Wautersiella; s__
Dendrobatesauratus-inhibitory_5	8				Panama	p__Proteobacteria; c__Gammaproteobacteria; o__Xanthomonadales; f__Xanthomonadaceae; g__Stenotrophomonas; s__
Espadarana prosoblepon-inhibitory_40	7				Panama	p__Proteobacteria; c__Gammaproteobacteria; o__Pseudomonadales; f__Pseudomonadaceae; g__Pseudomonas; s__
Lithobatescatesbeianus-inhibitory_37	7		16	16	Colorado	p__Actinobacteria; c__Actinobacteria; o__Actinomycetales; f__Microbacteriaceae; g__; s__
Litorianannotis-inhibitory_24	8		16	16	Queensland	p__Bacteroidetes; c__Sphingobacteriia; o__Sphingobacteriales; f__Sphingobacteriaceae; g__Sphingobacterium; s__multivorum
Litorianannotis-inhibitory_52†	7				Queensland	p__Proteobacteria; c__Gammaproteobacteria; o__Xanthomonadales; f__Xanthomonadaceae; g__Stenotrophomonas; s__
Litoriarheocola-inhibitory_25	8				Queensland	p__Proteobacteria; c__Alphaproteobacteria; o__Sphingomonadales; f__Sphingomonadaceae; g__Novosphingobium; s__
Smiliscasordida-inhibitory_34	7				Panama	p__Proteobacteria; c__Gammaproteobacteria; o__Pseudomonadales; f__Pseudomonadaceae; g__Pseudomonas
Strabomantisbufoniformis-inhibitory_11				16	Panama	p__Proteobacteria; c__Gammaproteobacteria; o__Aeromonadales; f__Aeromonadaceae; g__; s__

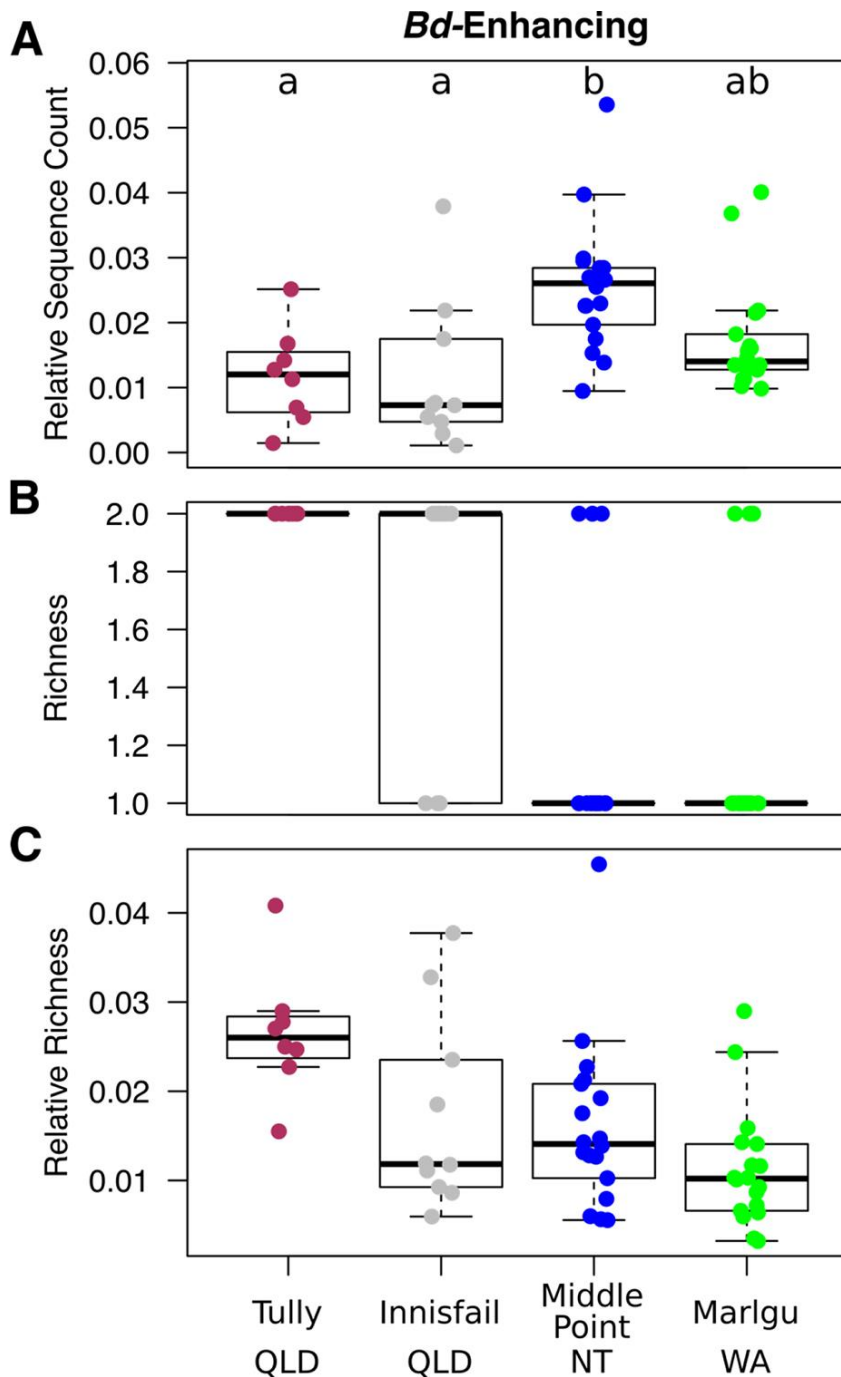


SampleID	TULLY, n = 8	INN, n = 10	NT, n = 18	WA, n = 18	Region	Uclust_taxonomy
Total <i>B. dendrobatidis</i> -inhibitory in core	13	4	2	5		

\*SampleID denotes the isolate name from the Antifungal Isolates Database. SampleID includes the frog species from which the bacterium was originally isolated. Region and Uclust\_taxonomy data are from the Woodhams et al. (7) metadata, indicating where in the world the bacterium was isolated for fungal-inhibition challenge experiments. Toad data are separated by sampling site: INN, Innisfail, QLD; TULLY, Tully, QLD; NT, Middle Point, NT; WA, Marigu Billabong, WA. Values indicate the number of samples with the bacterial type, where the bacterium was part of the "core" community (i.e., blank spaces do not equal zeros).  
†Bacterium found in negative controls.



**Appendix Figure 1.** Four toad sampling sites in northern Australia with years of invasive toad arrival to the sites.



**Appendix Figure 2.** Boxplots of proportion of sequences (A), total richness (B), and proportion of richness (C) represented by *Batrachochytrium*–enhancing bacteria. Points indicate values for individual toads. Boxplots indicate the median (thick line), interquartile range (the box), reasonable range of the data (dashed lines to the whiskers), and outliers. Letters above plots indicate significant differences from Tukey's post hoc tests with  $p < 0.05$ . *Bd*, *Batrachochytrium dendrobatidis*; NT, Northern Territory; QLD, Queensland; WA, Western Australia.